THE ROLE OF INTERFIBRILLAR PROTEOGLYCANS IN FORCE TRANSMISSION WITHIN TENDONS

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RELEVANCE TO MUSCULOSKELETAL CONDITIONS: This study addresses the contribution of interfibrillar proteoglycans to the tensile strength of tendon, a potentially important factor in the healing of tendon, ligament, and their graft replacements.

INTRODUCTION: Tendon and ligament are composite materials in which collagen fibrils are imbedded in a proteoglycan matrix. The collagen fibrils are believed to be shorter than the tissue length so a mechanism must exist to transmit loads between fibrils. Estimates of fibril length range from 1 to 6 mm [1,2] which is larger than their estimated critical length (~100μm), i.e. the length above which failure occurs through the fibrils rather than through the interfibrillar matrix [1]. Matrix proteoglycans, such as decorin, have been shown to be arranged periodically along the fibril length and appear to link adjacent fibrils [3]. The purpose of this study was to test the hypothesis that the interfibrillar proteoglycans are responsible for force transmission between fibrils and thus help determine tissue strength.

METHODS: Two hundred rat tail tendons from 13 female Sprague-Dawley rats were treated with three chemicals that selectively disrupt the interfibrillar proteoglycans. Fresh tendons were harvested according to protocol approved by the Institutional Animal Care and Use Committee. Group I (n=56) was treated with 4.0M guanidine hydrochloride (GuHCl) at 4°C and pH 7.4 for 15 hours in order to disrupt hydrophobic interactions. Group II (n=64) was treated with 2% 2-mercaptoethanol (2-ME) at 25°C and pH 7.2 for 16 hours in order to cleave disulfide bonds. Group III (n=80) was treated with chondroitinase ABC (C-ABC) at 37°C and pH 8.0 for 18 hours in order to cleave the $\beta_{1\text{-}4}$ glycosidic bonds in glycosaminoglycan side chains. The levels of C-ABC treatments were 0.018, 0.035, 0.070, 0.140 and 0.280 units/ml. Maximum failure force was determined following incubation by stretching the tendons at 10%/second until failure. Tensile tests of Group I were made 0.5, 2.5 and 6.5 hours after removal of the GuHCl. Analysis of variance was used to assess differences between the treatment and control tendons while maintaining an alpha of 0.05.

RESULTS: Both the GuHCl and the 2-ME treatments were found to significantly reduce tendon strength. Treatment with 4.0M GuHCl produced a 96% redcution in strength that was found to be reversible after treatment removal (Figure 1). Statistical analysis indicated that GuHCl treatment ($p<0.001$), time after removal of the treatment ($p<0.01$) and the treatment-time interaction ($p<0.015$) were all significant. Treatment with 2% 2-ME produced a 12% reduction in strength ($p<0.0001$). No concentration of C-ABC used in this study was found to affect failure force ($p=0.210$).

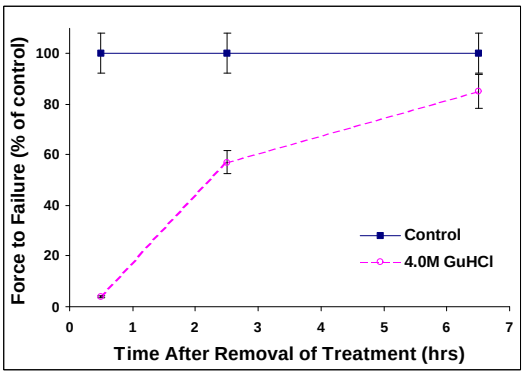

Figure 1: Effect of failure force on time after removal of treatment for treament and control tendons.

DISCUSSION: These observations are consistent with the hypothesis that the interfibrillar proteoglycans, specifically their protein cores, are intimately involved in force transmission between fibrils. The lack of effect of C-ABC combined with the effects produced by the GuHCl and the 2-ME suggest that interfribrillar force transmission primarily involves the protein core and not the GAG side chains. Further biochemical and electron microscopic studies are needed to verify these conclusions.

REFERENCES: 1) Parry, et al. In Collagn Vol II, 1-24. Nimni, editor, 1988, 2) Trotter, et al, Trans 35th ORS, 1989, 3) Scott, Biochemistry 35:8795-99, 1996.

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